



# Low-Dose Persistent Organic Pollutants Impair Insulin Secretory Function of Pancreatic $\beta$ -Cells: Human and In Vitro Evidence

Yu-Mi Lee,<sup>1</sup> Chae-Myeong Ha,<sup>2,3</sup> Se-A Kim,<sup>2,3</sup> Themis Thoudam,<sup>2,3</sup> Young-Ran Yoon,<sup>3,4</sup> Dae-Jung Kim,<sup>5</sup> Hyeon-Chang Kim,<sup>6</sup> Hyo-Bang Moon,<sup>7</sup> Sungmi Park,<sup>8</sup> In-Kyu Lee,<sup>3,8,9</sup> and Duk-Hee Lee<sup>1,3</sup>

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**Low-dose persistent organic pollutants (POPs), especially organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs), have emerged as a new risk factor for type 2 diabetes. We evaluated whether chronic exposure to low-dose POPs affects insulin secretory function of  $\beta$ -cells in humans and in vitro cells. Serum concentrations of OCPs and PCBs were measured in 200 adults without diabetes. Mathematical model-based insulin secretion indices were estimated by using a 2-h seven-sample oral glucose tolerance test. Insulin secretion by INS-1E  $\beta$ -cells was measured after 48 h of treatment with three OCPs or one PCB mixture. Static second-phase insulin secretion significantly decreased with increasing serum concentrations of OCPs. Adjusted means were 63.2, 39.3, 44.1, 39.3, 39.7, and 22.3 across six categories of a summary measure of OCPs ( $P_{\text{trend}} = 0.02$ ). Dynamic first-phase insulin secretion remarkably decreased with increasing concentrations of OCPs among only insulin-sensitive individuals ( $P_{\text{trend}} = 0.02$ ); the insulin levels among individuals with high OCPs were  $\sim 30\%$  of those with low OCPs. Compared with OCPs, PCBs showed weaker associations. The decreased insulin secretion by INS-1E  $\beta$ -cells was observed for even 1 pmol/L OCP. The data from human and in vitro cell experiments suggest that**

**chronic exposure to low-dose POPs, especially OCPs, can induce pancreatic  $\beta$ -cell dysfunction.**

Chronic exposure to low-dose chlorinated persistent organic pollutants (POPs), especially organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs), has been linked to the risk of type 2 diabetes (T2D) (1). POPs include several hundred highly lipophilic chemicals that bioaccumulate mainly in adipose tissue and are resistant to biodegradation (1). Although most chlorinated POPs were banned several decades ago in many countries, the modern general population is exposed to these chemicals through POP-contaminated foods, such as fatty animal foods, seafood, dairy products, and breast milk (1). Besides, these chemicals stored in adipose tissue are an important internal exposure source (1). The release of these chemicals from adipose tissue is mechanistically linked to adiposity, a traditional risk factor of T2D (1,2).

Both insulin resistance (IR) and  $\beta$ -cell dysfunction are important for the development of T2D, but many people exhibit predominantly either IR or  $\beta$ -cell dysfunction (3). Moreover, the role of obesity, the most important known

<sup>1</sup>Department of Preventive Medicine, School of Medicine, Kyungpook National University, Daegu, Republic of Korea

<sup>2</sup>Department of Biomedical Science, Graduate School, Kyungpook National University, Daegu, Republic of Korea

<sup>3</sup>BK21 Plus KNU Biomedical Convergence Program, Department of Biomedical Science, Kyungpook National University, Daegu, Republic of Korea

<sup>4</sup>Department of Biomedical Science, School of Medicine, Kyungpook National University and Hospital, Daegu, Republic of Korea

<sup>5</sup>Department of Endocrinology and Metabolism, Ajou University School of Medicine, Suwon, Republic of Korea

<sup>6</sup>Department of Preventive Medicine, Yonsei University College of Medicine, Seoul, Republic of Korea

<sup>7</sup>Department of Environmental Marine Sciences, College of Science and Technology, Hanyang University, Ansan, Republic of Korea

<sup>8</sup>Leading-edge Research Center for Drug Discovery and Development for Diabetes and Metabolic Disease, Kyungpook National University, Daegu, Republic of Korea

<sup>9</sup>Department of Internal Medicine, School of Medicine, Kyungpook National University, Daegu, Republic of Korea

Corresponding authors: Duk-Hee Lee, [lee\\_dh@knu.ac.kr](mailto:lee_dh@knu.ac.kr), and In-Kyu Lee, [leei@knu.ac.kr](mailto:leei@knu.ac.kr).

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Y.-M.L. and C.-M.H. contributed equally to this work.

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risk factor of T2D, seems to differ depending on the types of T2D preceded predominantly by IR versus  $\beta$ -cell dysfunction (4). Although overweight and obesity are the most important risk factors of IR-dominant T2D, the role of adiposity is weak in T2D preceded predominantly by  $\beta$ -cell dysfunction (4). At present, genetic predisposition is considered a key determinant of  $\beta$ -cell function (5).

Although epidemiological and experimental studies have revealed the relationship of POPs with IR (6,7), some human studies have suggested that direct toxicity of POPs to  $\beta$ -cells (rather than to decreased peripheral insulin sensitivity) may be a more plausible mechanism linking POPs and T2D (8–10). For example, serum concentrations of POPs were related to the markers of  $\beta$ -cell dysfunction (e.g., HOMA- $\beta$  and 2-h insulin after oral glucose tolerance test [OGTT]), not IR markers among Greenland Inuit without diabetes (9). In addition, serum concentrations of PCBs were related to low levels of fasting insulin or HOMA- $\beta$ , not IR, among children (8,10). On the other hand, in one study that used an intravenous glucose tolerance test, *p,p'*-dichlorodiphenyltrichloroethane (DDT), not other POPs, was associated with both HOMA-IR and first-phase insulin secretion after adjustment for peripheral insulin sensitivity (11). Because decreased insulin secretion as a result of pancreatic  $\beta$ -cell dysfunction is crucial for the development of overt T2D (12), careful evaluation of the association between POPs and insulin secretion is important to understand the role of POPs in the pathogenesis of T2D.

Although hyperglycemic-euglycemic insulin clamp techniques are widely considered gold standard methods for assessing insulin secretion and IR in vivo (13), these approaches are difficult to apply practically to epidemiological studies. Several surrogate measures are derived from OGTTs or fasting insulin and glucose, but all these methods have limitations, and correlations among the indices are modest (14).

We assessed mathematical model-based insulin secretion (dynamic first- and static second-phase of  $\beta$ -cell function) and insulin sensitivity among participants without diabetes by using a 2-h seven-sample OGTT, a reduced version of a 5-h 11-sample full oral minimal model assessment that was validated against clamp methods (15,16), and evaluated the relationship with serum concentrations of OCPs or PCBs. In addition, we determined whether the findings in humans were reproduced in in vitro cell experiments.

## RESEARCH DESIGN AND METHODS

### Study Participants

Two hundred patients were recruited at the Routine Health Checkup Center of Kyungpook National University Hospital, Daegu, Korea, from October 2013 to December 2015. Among patients age  $>30$  years, those with at least one of the following criteria were excluded: 1) any history of diabetes, myocardial infarction, stroke, or heart failure; 2) diagnosis of cancer within previous 5 years; 3) current participation in any clinical drug trial; or 4) pregnancy.

We obtained written informed consent from each participant. The study's protocol was reviewed and approved by the Institutional Review Board of Kyungpook National University Hospital (IRB No. KNUH 2013-12-016).

### Measurements

Detailed information on general characteristics and health-related behaviors is provided in the Supplementary Data. Before venous blood sampling, all the participants fasted overnight for at least 8 h. Approximately 5 mL of serum was drawn from each participant, and the samples were kept in a freezer at  $-70^{\circ}\text{C}$  until analysis. Total cholesterol and triglyceride levels were measured by an enzymatic method by using an ADVIA 1800 autoanalyzer (Siemens Medical Solutions, Malvern, PA). Glucose was measured by a colorimetric method on the same analyzer. Insulin and C-peptide were quantified by a radioimmunoassay method (SR-300; STRATEC, Birkenfeld, Germany). Intra- and interassay coefficients of variation were 0.5 and 0.8 for glucose, 1.8 and 6.3 for insulin, and 2.7 and 4.0 for C-peptide, respectively.

### Two-Hour Seven-Sample OGTT

For the minimal model assessment of pancreatic  $\beta$ -cell responsiveness, we used a 2-h OGTT protocol that consisted of seven venous blood samplings at 0, 10, 20, 30, 60, 90, and 120 min after ingestion of 75 g of glucose at time 0 (15). Plasma glucose, insulin, and C-peptide levels were measured for each sample.

### Indices of Insulin Sensitivity and Insulin Secretion of Pancreatic $\beta$ -Cells

Indices of insulin sensitivity and insulin secretion were obtained from seven samples of glucose, insulin, and C-peptide concentrations measured during the OGTT protocol by using the minimal model (17–19) and were calculated by means of SAAM II (Simulation Analysis and Modeling) version 2.1 software (<https://tegvirginia.com/solutions/saam-ii>) and the individual estimates from the 2-h seven-sample OGTT. The insulin sensitivity index is the ability of insulin to stimulate glucose disposal and inhibit glucose production (20). Insulin secretion indices consist of two components: dynamic phase secretion ( $\Phi_d$ ) and static phase secretion ( $\Phi_s$ ) (15);  $\Phi_d$  is the amount of dynamic first-phase secretion of insulin per unit increase of glucose, which is used to assess the appropriateness of insulin secretion in response to a change in glucose;  $\Phi_s$  is over-basal average static second-phase secretion of insulin per unit over-basal average glucose, which is used to assess the appropriateness of insulin secretion for a given glucose level (20). The disposition index (DI) is a product of the insulin sensitivity index ( $S_I$ ) and  $\beta$ -cell insulin secretion. Thus, dynamic phase DI ( $DI_d$ ) =  $\Phi_d \times S_I$ , and static phase DI ( $DI_s$ ) =  $\Phi_s \times S_I$  (20).

In addition, we calculated conventional estimators HOMA-IR and HOMA- $\beta$  to assess insulin sensitivity and insulin release in most epidemiological studies. The equations are as follows: HOMA-IR = (fasting insulin [ $\mu\text{IU/mL}$ ]  $\times$  fasting

plasma glucose [mmol/L])/22.5 and  $\text{HOMA-}\beta = (20 \times \text{fasting plasma insulin } [\mu\text{IU/mL}]) / (\text{fasting plasma glucose [mmol/L]} - 3.5)$  (21).

### Measurement of POPs in Serum

Serum concentrations of POPs, including PCBs and OCPs, were analyzed in the laboratory of Hanyang University (Ansan, Korea) by using high-resolution gas chromatography with high-resolution mass spectrometry (AutoSpec Premier; Waters, Milford, MA). POP concentrations in serum were reported as the wet weight in picograms per milliliter. The limit of detection (LOD) was defined as the concentration that generated a signal equal to three times the baseline noise. In the analysis, POP concentrations below the LOD were replaced with  $\text{LOD} / 3$ . Thirty-seven POPs (18 OCPs, 19 PCBs) were quantified, but we evaluated 4 OCPs and 6 PCBs for which at least 70% of the participants had concentrations above the LOD: *p,p'*-DDT; *p,p'*-dichlorodiphenyldichloroethylene (DDE);  $\beta$ -hexachlorocyclohexane (HCH); *trans*-nonachlor; PCB118; PCB138; PCB153; PCB170; PCB180; and PCB187. We tried both wet-weight concentrations with lipid adjustment (including serum concentrations of triglycerides and total cholesterol as covariates) and lipid-standardized concentrations (by dividing wet-weight concentrations by total lipids [total lipids (mg/dL) =  $2.27 \times \text{total cholesterol} + \text{triglycerides} + 62.3$ ]) (22). Because they showed similar associations, we present the results of wet-weight concentrations.

Supplementary Table 1 shows the detection rate and distribution of POP concentrations. For a direct comparison of results with the *in vitro* cell study, we present concentrations of POPs in molar units (nmol/L) in cases of  $\beta$ -HCH, *p,p'*-DDT, *trans*-nonachlor, and the mixture of PCBs.

### In Vitro Experiments

Insulin secretion and insulin content were analyzed to assess the chronic effects of three low-dose OCPs (*p,p'*-DDT;  $\beta$ -HCH; and *trans*-nonachlor) and Aroclor 1254 (PCB mixture) on INS-1E  $\beta$ -cells. For insulin assays,  $3.0 \times 10^5$  INS-1E cells per well were seeded in 24-well plates with complete RPMI medium and grown for 48 h. The cells were preincubated in the RPMI medium containing 0.5% of FBS for 1 h followed by incubation with various concentrations of each compound ( $10^{-12}$ – $10^{-6}$  mol/L) for 48 h. The OCPs and PCB mixture were dissolved and stored in absolute methanol. The final concentration of methanol in the media was 0.1% volume for volume, including the control. Measurement of glucose-stimulated insulin secretion was performed as previously described (23,24) with some modifications. Detailed information on *in vitro* experiments is provided in the Supplementary Data.

### Statistical Analysis

Serum concentrations of individual POPs were categorized into quartiles, and the last quartile was further categorized with the cutoff points of 90th and 95th percentile levels because the range of the last quartile was too wide, and insulin secretion clearly decreased

with increasing concentrations of POPs, even within the 4th quartile (Q4). In addition, we determined the summary measures of OCPs and PCBs by summing the ranks of the individual congeners of four OCPs (*p,p'*-DDT; *p,p'*-DDE;  $\beta$ -HCH; and *trans*-nonachlor) and six PCBs (PCB118, PCB138, PCB153, PCB170, PCB180, and PCB187), respectively. The summary measures were also categorized into groups by cutoff points at the 25th, 50th, 75th, 90th, and 95th percentile levels. Distributions of each compound according to the categories of the summary measures of OCPs and PCBs are presented in Supplementary Table 2.

In fact, human studies on POPs should be interpreted by primarily focusing on the summary measures of POPs rather than on individual compounds because the serum concentrations of individual compounds highly correlate in the general population (correlation coefficients among four OCPs and six PCBs in this study: 0.35–0.87 and 0.76–0.98, respectively). In this situation, interpretation that focuses on individual compounds may be misleading. Among several methods for estimation of the summary measures of POPs (1), we used the summary measure that sums the rank orders of individual compounds belonging to each subclass; this approach enables equal contributions from all constituent compounds. Absolute concentration-based summary measures seem to be intuitively reasonable, but their results are similar to those of a couple of individual compounds with much higher absolute concentrations compared with the other compounds. The advantage of a rank-based summary measure of POPs over other methods is discussed in detail elsewhere (1).

Insulin secretion and sensitivity indices were log-transformed, controlling for the skewed distribution. Adjusted geometric means of insulin secretion and sensitivity indices according to POP concentrations were estimated by using the generalized linear model. The covariates were age (continuous, years), sex (male/female), BMI (continuous,  $\text{kg/m}^2$ ), cigarette smoking (continuous, pack-years), alcohol consumption (continuous, g/week), physical activity (continuous, MET-min/week), total cholesterol (continuous, mg/dL), and triglycerides (continuous, mg/dL). When the indices of insulin secretion were an outcome variable, insulin sensitivity was also considered as a covariate or an effect modifier because insulin secretion from pancreatic  $\beta$ -cells is directly affected by insulin sensitivity. Correlation coefficients of covariates with insulin sensitivity/insulin secretion indices are provided in Supplementary Table 3. All data were analyzed with SAS 9.4 software (SAS Institute, Cary, NC).

## RESULTS

### Human Study

Table 1 shows general characteristics of the study participants. Among the 200 participants, 36% were male. The mean age was 55.1 years, and the mean BMI was  $24.6 \text{ kg/m}^2$ . Current smokers and current drinkers constituted 11.5% and 49.5% of the cohort, respectively. Correlation coefficients of BMI and health behaviors with the summary measures of OCPs and PCBs are shown in

**Table 1—General characteristics of study participants**

Characteristic	Value
Age (years)	55.1 $\pm$ 12.1 (30–71)
Sex	
Male	72 (36.0)
Female	128 (64.0)
BMI (kg/m <sup>2</sup> )	24.6 $\pm$ 3.3 (17.0–37.4)
<25	117 (58.5)
$\geq$ 25 to <30	71 (35.5)
$\geq$ 30	12 (6.0)
Cigarette smoking (pack-years)	6.7 $\pm$ 13.6 (0–60)
Alcohol consumption (g/week)	79.0 $\pm$ 223.9 (0–2,580)
Physical activity (MET-min/week)	2,749.2 $\pm$ 3,796.3 (0–24,318.0)
Total cholesterol (mg/mL)	185.7 $\pm$ 32.4 (114.0–313.0)
Triglycerides (mg/mL)	128.2 $\pm$ 78.4 (34.0–536.0)

Data are mean  $\pm$  SD (minimum–maximum) or *n* (%).

Supplementary Table 4. BMI showed a weak positive correlation with the summary measure of OCPs but not with the summary measure of PCBs.

Markers of insulin sensitivity and secretion estimated on the basis of 2-h seven-sample OGTT were only weakly associated with HOMA-IR and HOMA- $\beta$ , which were estimated on the basis of fasting glucose and insulin (Supplementary Table 5). Correlation coefficients between static and dynamic insulin secretion estimated by means of 2-h seven-sample OGTT with HOMA- $\beta$  were 0.26 and 0.30, respectively.

Table 2 shows the association between summary measures of OCPs or PCBs and markers of insulin secretion and sensitivity indices among all the participants. Results on individual compounds belonging to OCPs and PCBs are presented in Supplementary Tables 6 and 7. In crude models, insulin sensitivity decreased as the summary measure of OCPs or PCBs increased ( $P_{\text{trend}} < 0.01$  for OCPs and 0.01 for PCBs). Among the markers of insulin secretion,  $\Phi_s$  significantly decreased as the summary measure of OCPs or PCBs increased ( $P_{\text{trend}} < 0.01$  for OCPs and 0.03 for PCBs), whereas  $\Phi_d$  was not statistically significant despite a decreasing tendency with increasing dose of OCPs or PCBs.

After adjustment for age, sex, BMI, cigarette smoking, alcohol consumption, physical activity, total cholesterol, and triglycerides (model 2), only the inverse relation between OCPs and  $\Phi_s$  remained significant; adjusted geometric means of  $\Phi_s$  were 63.4, 38.4, 44.2, 39.4, 40.1, and 22.7 across six categories of summary measures of OCPs (<25%, 25 to <50%, 50 to <75%, 75 to <90%, 90 to <95%,  $\geq$ 95%;  $P_{\text{trend}} = 0.03$ ). When insulin sensitivity was further adjusted (model 3), the relation remained unchanged. OCPs also showed a decreasing tendency with static DI, with a marginal statistical significance ( $P_{\text{trend}} =$

0.06). On the other hand, when HOMA-IR and HOMA- $\beta$  were used as markers of insulin sensitivity and insulin secretion, the summary measure of PCBs was inversely associated with HOMA- $\beta$  ( $P_{\text{trend}} = 0.02$  in model 3), whereas OCPs were not related to either.

Evaluation of the association of OCPs or PCBs with glucose during fasting and at 2 h showed that glucose levels at 2 h gradually increased with increasing serum concentrations of OCPs and PCBs, whereas the increase in fasting glucose was prominent in the highest 5th percentile group of OCPs or PCBs (Supplementary Table 8). We repeated all these analyses after excluding those with concentrations below the LOD as a sensitivity analysis, and the results were very similar to those among all participants (Supplementary Table 9).

We next assessed the associations of POPs with insulin secretion by the levels of IR (Fig. 1) and found a statistically significant interaction between the summary measure of OCPs and HOMA-IR on  $\Phi_d$  ( $P_{\text{interaction}} = 0.01$ ). Among participants with HOMA-IR <1.95 (median value; relatively insulin-sensitive individuals), a strong inverse association was observed between OCPs and  $\Phi_d$  ( $P_{\text{trend}} = 0.02$ ); the adjusted mean of insulin secretion among participants in Q4 of OCPs was <30% of that in the Q1 of OCPs. In contrast, no association was found between OCPs and  $\Phi_d$  among participants with HOMA-IR  $\geq$ 1.95 (relatively insulin-resistant individuals). Similarly, the  $\Phi_s$  also showed a tendency for a stronger inverse association with OCPs among relatively insulin-sensitive individuals compared with relatively insulin-resistant individuals, although the *P* value for the interaction was not statistically significant (Fig. 1).

Results for PCBs were generally weaker than those for OCPs (Fig. 2). PCBs showed a clearer pattern of interaction with HOMA- $\beta$  ( $P_{\text{interaction}} = 0.09$ ) than with markers of insulin secretion on the basis of the 2-h seven-sample OGTT ( $P_{\text{interaction}} = 0.69$  for  $\Phi_s$  and 0.12 for  $\Phi_d$ ). As in the OCP findings, the decreasing trend of insulin secretion was clearer among relatively insulin-sensitive individuals than among relatively insulin-resistant individuals.

When we tried tertiles of HOMA-IR in sensitivity analyses and participants were restricted to the relatively insulin-sensitive individuals, the patterns became more prominent (Supplementary Figs. 1 and 2). Of note, PCBs also showed a marginally significant interaction with  $\Phi_d$  ( $P_{\text{interaction}} = 0.07$ ). On the other hand, when the marker of insulin sensitivity, which was estimated by the 2-h seven-sample OGTT, was used instead of HOMA-IR, the interactions were not observed. The results of individual compounds belonging to OCPs and PCBs are presented in Supplementary Tables 10 and 11.

When we evaluated the associations of obesity indices (BMI, waist circumference, and percent body fat) with insulin secretion and sensitivity indices without consideration of POPs, only insulin sensitivity clearly decreased with increasing obesity (Table 3). Neither  $\Phi_s$  nor  $\Phi_d$  was associated with obesity indices.

Table 2—Geometric means of insulin sensitivity and secretion indicators according to categories of summary measures of OCPs and PCBs

	Summary measure of OCPs or PCBs					$P_{\text{trend}}$
	Q1 (<25%)	Q2 (25 to <50%)	Q3 (50 to <75%)	Q4-1 (75 to <90%)	Q4-2 (90 to <95%)	Q4-3 (≥95%)
$\Sigma$ OCPs*	75–234 $n = 49$	237–399 $n = 51$	401–561 $n = 51$	564–683 $n = 29$	686–720 $n = 10$	722–780 $n = 10$
$\Sigma$ PCBs†	38–318 $n = 50$	323–589 $n = 49$	593–872 $n = 51$	878–1,054 $n = 30$	1,061–1,125 $n = 10$	1,126–1,199 $n = 10$
$S_i$ ( $10^{-4}$ dL/kg/min per $\mu$ U/mL)						
$\Sigma$ OCPs						
Model 1	12.60 $\pm$ 1.85	8.47 $\pm$ 1.22	7.11 $\pm$ 1.02	6.68 $\pm$ 1.28	4.55 $\pm$ 1.48	5.44 $\pm$ 1.77
Model 2	10.06 $\pm$ 2.00	8.07 $\pm$ 1.11	7.45 $\pm$ 1.10	6.99 $\pm$ 1.40	6.12 $\pm$ 2.03	7.05 $\pm$ 2.31
$\Sigma$ PCBs						
Model 1	12.10 $\pm$ 1.77	8.75 $\pm$ 1.29	6.36 $\pm$ 0.92	6.15 $\pm$ 1.16	8.33 $\pm$ 2.72	6.98 $\pm$ 2.28
Model 2	9.21 $\pm$ 1.58	8.57 $\pm$ 1.20	6.64 $\pm$ 0.91	7.20 $\pm$ 1.39	11.00 $\pm$ 3.39	7.84 $\pm$ 2.49
$\Phi_s$ ( $10^{-9}$ /min)						
$\Sigma$ OCPs						
Model 1	63.45 $\pm$ 6.97	39.81 $\pm$ 4.29	44.61 $\pm$ 4.80	39.24 $\pm$ 5.60	44.35 $\pm$ 10.79	23.36 $\pm$ 5.68
Model 2	63.43 $\pm$ 10.20	38.42 $\pm$ 4.27	44.16 $\pm$ 5.30	39.42 $\pm$ 6.39	40.11 $\pm$ 10.79	22.71 $\pm$ 6.02
Model 3	63.15 $\pm$ 10.00	39.25 $\pm$ 4.31	44.12 $\pm$ 5.22	39.31 $\pm$ 6.28	39.67 $\pm$ 10.52	22.31 $\pm$ 5.83
$\Sigma$ PCBs						
Model 1	53.91 $\pm$ 6.06	47.11 $\pm$ 5.35	41.29 $\pm$ 4.60	40.58 $\pm$ 5.89	36.70 $\pm$ 9.23	35.81 $\pm$ 9.01
Model 2	47.01 $\pm$ 6.79	43.01 $\pm$ 5.08	43.50 $\pm$ 5.02	44.03 $\pm$ 7.15	39.14 $\pm$ 10.14	39.58 $\pm$ 10.58
Model 3	46.76 $\pm$ 6.65	43.80 $\pm$ 5.10	44.11 $\pm$ 5.01	43.82 $\pm$ 7.00	37.46 $\pm$ 9.58	38.75 $\pm$ 10.21
$\Phi_d$ ( $10^{-9}$ )						
$\Sigma$ OCPs						
Model 1	484.88 $\pm$ 92.64	509.32 $\pm$ 95.39	380.51 $\pm$ 71.26	408.65 $\pm$ 101.49	460.74 $\pm$ 194.87	393.50 $\pm$ 166.43
Model 2	547.47 $\pm$ 155.58	485.15 $\pm$ 95.29	356.73 $\pm$ 75.71	380.93 $\pm$ 109.12	338.51 $\pm$ 160.99	342.72 $\pm$ 160.44
Model 3	545.71 $\pm$ 155.05	492.76 $\pm$ 97.05	356.50 $\pm$ 75.64	380.19 $\pm$ 108.89	335.80 $\pm$ 159.69	338.33 $\pm$ 158.41
$\Sigma$ PCBs						
Model 1	549.84 $\pm$ 103.58	465.01 $\pm$ 88.49	368.79 $\pm$ 68.79	403.21 $\pm$ 98.06	559.83 $\pm$ 235.82	338.46 $\pm$ 142.57
Model 2	655.84 $\pm$ 160.90	427.45 $\pm$ 85.77	367.85 $\pm$ 72.10	329.79 $\pm$ 90.98	482.11 $\pm$ 212.39	269.08 $\pm$ 122.27
Model 3	653.64 $\pm$ 160.44	432.24 $\pm$ 86.92	371.02 $\pm$ 72.83	328.80 $\pm$ 90.75	469.27 $\pm$ 207.26	265.57 $\pm$ 120.78
$DI_s$ ( $10^{-13}$ dL/kg/min <sup>2</sup> per $\mu$ U/mL)						
$\Sigma$ OCPs						
Model 1	669.26 $\pm$ 159.22	260.94 $\pm$ 60.85	230.01 $\pm$ 53.64	184.58 $\pm$ 57.08	136.88 $\pm$ 72.08	85.42 $\pm$ 44.98
Model 2	521.83 $\pm$ 172.10	239.20 $\pm$ 54.53	238.96 $\pm$ 58.86	193.60 $\pm$ 64.36	171.12 $\pm$ 94.44	110.62 $\pm$ 60.10
$\Sigma$ PCBs						
Model 1	542.01 $\pm$ 130.37	321.21 $\pm$ 78.05	181.92 $\pm$ 43.33	179.72 $\pm$ 55.81	228.11 $\pm$ 122.69	179.46 $\pm$ 96.52
Model 2	337.86 $\pm$ 97.94	285.60 $\pm$ 67.71	202.41 $\pm$ 46.88	238.87 $\pm$ 77.87	338.51 $\pm$ 176.21	233.89 $\pm$ 125.58

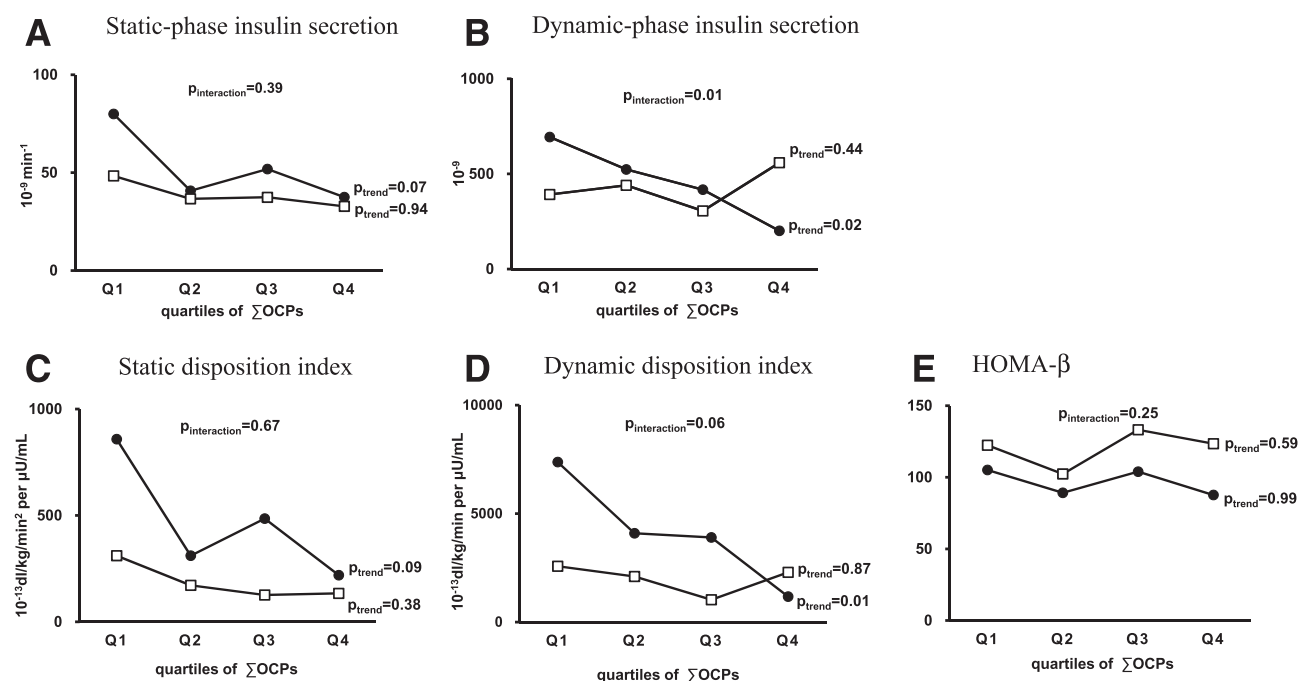
Continued on p. 2674

Table 2—Continued

	Summary measure of OCPs or PCBs					$P_{\text{trend}}$
	Q1 (<25%)	Q2 (25 to <50%)	Q3 (50 to <75%)	Q4-1 (75 to <90%)	Q4-2 (90 to <95%)	Q4-3 ( $\geq 95\%$ )
$DI_d (10^{-13} \text{ dL/kg/min per } \mu\text{U/mL})$						
$\Sigma\text{OCPs}$						
Model 1	5,151.39 $\pm$ 1,411.89	3,394.62 $\pm$ 911.97	1,953.81 $\pm$ 524.89	1,927.51 $\pm$ 686.71	1,409.22 $\pm$ 854.97	1,468.69 $\pm$ 891.06
Model 2	4,534.08 $\pm$ 1,806.82	3,067.63 $\pm$ 844.95	1,929.15 $\pm$ 574.13	1,880.66 $\pm$ 755.48	1,426.09 $\pm$ 951.08	1,708.55 $\pm$ 1,121.63
$\Sigma\text{PCBs}$						
Model 1	5,596.03 $\pm$ 1,508.06	3,203.52 $\pm$ 872.08	1,622.86 $\pm$ 433.03	1,774.31 $\pm$ 617.30	3,555.82 $\pm$ 2,142.72	1,713.70 $\pm$ 1,032.67
Model 2	4,770.43 $\pm$ 1,637.20	2,862.60 $\pm$ 803.54	1,717.23 $\pm$ 470.83	1,777.56 $\pm$ 686.00	4,276.38 $\pm$ 2,635.38	1,614.27 $\pm$ 1,026.14
HOMA-IR						
$\Sigma\text{OCPs}$						
Model 1	3.06 $\pm$ 0.14	2.74 $\pm$ 0.12	3.02 $\pm$ 0.13	3.40 $\pm$ 0.20	2.83 $\pm$ 0.28	3.85 $\pm$ 0.38
Model 2	3.19 $\pm$ 0.18	2.77 $\pm$ 0.11	3.04 $\pm$ 0.13	3.42 $\pm$ 0.20	2.52 $\pm$ 0.24	3.61 $\pm$ 0.34
Model 3	3.19 $\pm$ 0.18	2.76 $\pm$ 0.11	3.04 $\pm$ 0.13	3.42 $\pm$ 0.20	2.53 $\pm$ 0.24	3.62 $\pm$ 0.34
$\Sigma\text{PCBs}$						
Model 1	3.15 $\pm$ 0.14	2.75 $\pm$ 0.13	3.05 $\pm$ 0.14	3.16 $\pm$ 0.18	3.45 $\pm$ 0.35	3.10 $\pm$ 0.31
Model 2	3.26 $\pm$ 0.17	2.72 $\pm$ 0.11	3.07 $\pm$ 0.13	3.13 $\pm$ 0.18	3.24 $\pm$ 0.30	3.25 $\pm$ 0.31
Model 3	3.27 $\pm$ 0.17	2.71 $\pm$ 0.11	3.07 $\pm$ 0.13	3.13 $\pm$ 0.18	3.26 $\pm$ 0.30	3.26 $\pm$ 0.31
HOMA- $\beta$						
$\Sigma\text{OCPs}$						
Model 1	128.62 $\pm$ 8.84	99.42 $\pm$ 6.70	112.31 $\pm$ 7.57	105.80 $\pm$ 9.45	97.63 $\pm$ 14.86	92.21 $\pm$ 14.03
Model 2	116.02 $\pm$ 10.68	95.90 $\pm$ 6.10	118.45 $\pm$ 8.15	114.93 $\pm$ 10.67	91.66 $\pm$ 14.13	92.45 $\pm$ 14.02
Model 3	116.13 $\pm$ 10.69	95.43 $\pm$ 6.09	118.47 $\pm$ 8.15	115.00 $\pm$ 10.67	91.89 $\pm$ 14.16	92.83 $\pm$ 14.08
$\Sigma\text{PCBs}$						
Model 1	130.83 $\pm$ 8.77	114.72 $\pm$ 7.77	103.27 $\pm$ 6.85	99.70 $\pm$ 8.63	94.41 $\pm$ 14.15	77.09 $\pm$ 11.55
Model 2	127.10 $\pm$ 10.26	107.57 $\pm$ 7.10	106.99 $\pm$ 6.90	101.27 $\pm$ 9.19	89.22 $\pm$ 12.94	82.87 $\pm$ 12.39
Model 3	127.22 $\pm$ 10.28	107.23 $\pm$ 7.10	106.73 $\pm$ 6.90	101.36 $\pm$ 9.21	89.91 $\pm$ 13.08	83.18 $\pm$ 12.46

Data are mean  $\pm$  SD unless otherwise indicated. Model 1: crude. Model 2: adjusted for age, sex, BMI, cigarette smoking, alcohol consumption, physical activity, total cholesterol, and triglycerides. Model 3: further adjusted for insulin sensitivity. \* $\Sigma\text{OCPs}$ : rank sum of four OCPs (*p,p'*-DDT, *p,p'*-DDE,  $\beta$ -HCH, and *trans*-nonachlor). † $\Sigma\text{PCBs}$ : rank sum of six PCBs (PCB118, PCB138, PCB153, PCB170, PCB180, and PCB187).





**Figure 1**—Associations between insulin secretion indicators (A–E) and concentrations of OCPs stratified by the level of HOMA-IR.  $\Sigma\text{OCPs}$ : rank sum of four OCPs (*p,p'*-DDT; *p,p'*-DDE;  $\beta$ -HCH; and *trans*-nonachlor) adjusted for age, sex, BMI, cigarette smoking, alcohol consumption, physical activity, total cholesterol, triglycerides, and insulin sensitivity. For the analyses in which the static and dynamic DIs are dependent variables, insulin sensitivity was excluded from the preceding covariates. ●, insulin-sensitive group (HOMA-IR <1.95); □, insulin-resistant group (HOMA-IR  $\geq$ 1.95).

## In Vitro Experiments

The decreased insulin secretion after stimulation by glucose was observed for  $10^{-12}$  mol/L *p,p'*-DDT and  $\beta$ -HCH (Fig. 3A). At  $10^{-9}$  mol/L, insulin secretion levels were significantly decreased for all OCPs and Aroclor 1254. However, in the range of doses differing by  $\sim 10^6$ -fold ( $10^{-12}$ – $10^{-6}$  mol/L), no linear dose-response relations were observed, but nonmonotonic dose-response relations with some fluctuations were. Little or no effect of OCPs or Aroclor 1254 on the basal insulin secretion level was found (Supplementary Fig. 3).

Insulin content also significantly decreased in cells treated with *p,p'*-DDT ( $10^{-12}$ – $10^{-6}$  mol/L, except  $10^{-11}$  mol/L); *trans*-nonachlor ( $10^{-10}$ – $10^{-6}$  mol/L, except  $10^{-8}$  mol/L); and Aroclor 1254 ( $10^{-7}$ – $10^{-6}$  mol/L) (Fig. 3B). In contrast, the insulin content in  $\beta$ -HCH-treated cells significantly increased at low concentrations ( $10^{-12}$ – $10^{-11}$  mol/L). A very low concentration ( $10^{-11}$  mol/L) of Aroclor 1254 also yielded an increased concentration of intracellular insulin content. Again, no linear dose-response relations were found.

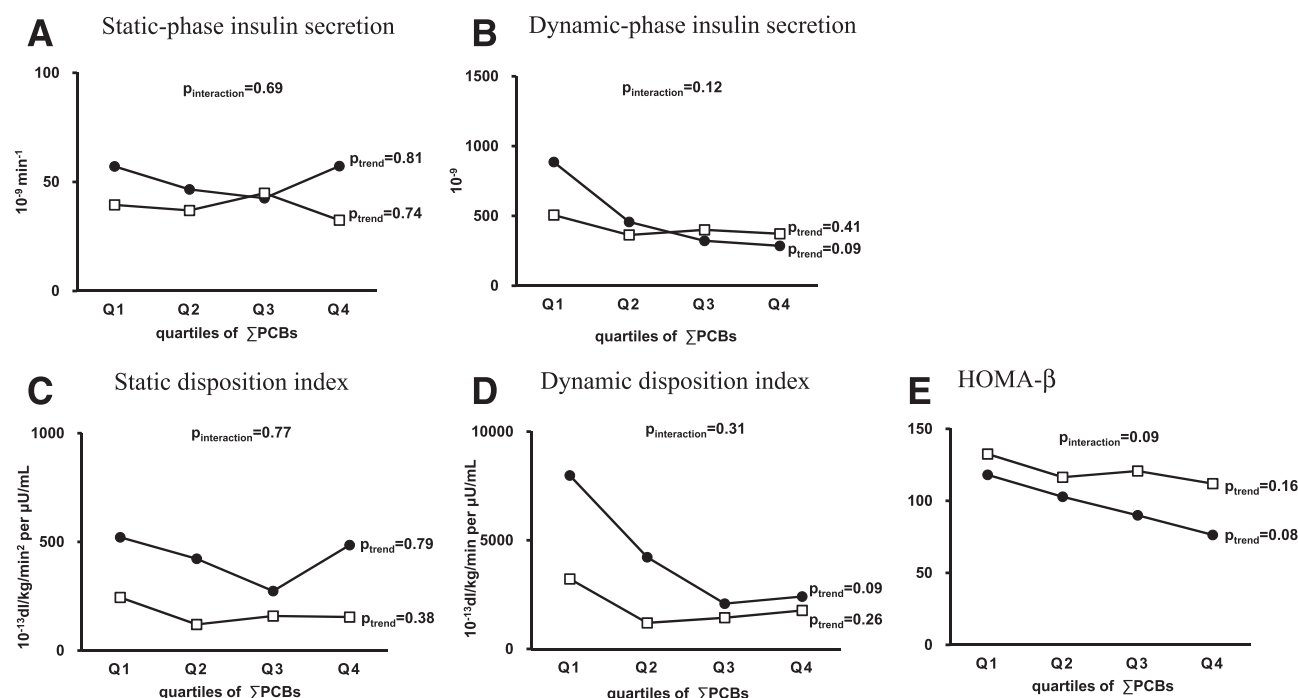
The ratio of insulin secretion/insulin content revealed different mechanisms of action among different chemical compounds (Fig. 3C). For example, *p,p'*-DDT showed the most consistent decrease pattern for insulin secretion and intracellular insulin content in INS-1E cells. Both  $\beta$ -HCH and Aroclor 1254 seemed to primarily decrease insulin secretion. In the case of *trans*-nonachlor, decreased insulin

synthesis seemed to be more pronounced than decreased insulin secretion.

## DISCUSSION

On the basis of human and in vitro studies, we demonstrate that chronic exposure to low-dose POPs can induce pancreatic  $\beta$ -cell dysfunction. POPs were found to be more strongly related to markers of insulin secretion than to IR. Between two common subclasses of POPs, OCPs showed more consistent results compared with PCBs. For example,  $\Phi_s$  among participants in the highest 5th percentile of the summary measure of OCPs was only one-third of those in the lowest 25th percentile. In contrast to POPs, obesity indices, such as BMI, waist circumference, and percent body fat, were found to be strongly associated only with IR but not with indices of insulin secretion.

Of note, the associations of POPs with  $\Phi_d$  were different depending on the levels of HOMA-IR. The inverse associations between these chemicals with  $\Phi_d$  were observed among insulin-sensitive individuals and not in insulin-resistant individuals. Among insulin-sensitive individuals, the insulin levels among those in Q4 of OCPs were only 20–30% of those in Q1 of these chemicals. However, these strong inverse associations completely disappeared as HOMA-IR increased. The  $\Phi_s$  also showed a similar interaction, but the pattern was weaker than that of  $\Phi_d$ . All these interactions were more pronounced when the analysis was restricted to more insulin-sensitive individuals.



**Figure 2**—Associations between insulin secretion indicators (A–E) and concentrations of PCBs stratified by the level of HOMA-IR.  $\Sigma$ PCBs: rank sum of six PCBs (PCB118, PCB138, PCB153, PCB170, PCB180, and PCB187) adjusted for age, sex, BMI, cigarette smoking, alcohol consumption, physical activity, total cholesterol, triglycerides, and insulin sensitivity. For the analyses in which the static and dynamic DIs are dependent variables, insulin sensitivity was excluded from the preceding covariates. ●, insulin-sensitive group (HOMA-IR <1.95); □, insulin-resistant group (HOMA-IR  $\geq$ 1.95).

As people become insulin resistant, a compensatory oversecretion of insulin occurs as long as the pancreatic  $\beta$ -cells work. Therefore, no association of POPs with  $\Phi_d$  among insulin-resistant individuals suggests that the inverse association between POPs and insulin secretion is masked by the hyperactivation of pancreatic  $\beta$ -cells. This notion indicates that the detrimental effect of chronic exposure to low-dose POPs on pancreatic  $\beta$ -cells may be the result of reversible functional impairment (which can be overcome by the compensatory mechanism to activate pancreatic  $\beta$ -cells) as opposed to the direct cellular toxicity resulting from high-dose chemical exposure that leads to irreversible cell damage.

The possibility of the reversible functional impairment of pancreatic  $\beta$ -cells is also supported by the findings from in vitro experiments. In this study, the treatment of INS-1E  $\beta$ -cells with 1 pmol/L *p,p'*-DDT for 48 h decreased the insulin secretion and intracellular insulin content, but no further decrease was observed with an increase in the dose of *p,p'*-DDT to 1  $\mu$ mol/L. Other compounds also demonstrated nonlinearity. Because direct cellular toxicity commonly shows a linear dose-response relation, it may not be a plausible mechanism underlying the decreased insulin secretion or intracellular insulin content resulting from POPs.

Low levels of POPs may indirectly affect the function of INS-1E  $\beta$ -cells, and this pathway can be compensated by an increased dose of POPs through certain biological

mechanisms. The possibility of nonlinearity was suggested in our review article summarizing various epidemiological and experimental studies of POPs and T2D (1). Of note, mitochondrial dysfunction, a possible molecular mechanism linking POPs and T2D (1,7,25), can be compensated by the induction of cellular protective mechanisms by slightly increased doses of chemicals; this phenomenon is called hormesis (26).

In addition, the current in vitro experiments suggest that different chemical compounds have different molecular mechanisms of action. For example, *p,p'*-DDT showed the most consistent pattern for decreased insulin secretion and a decrease in intracellular insulin content. Both  $\beta$ -HCH and Aroclor 1254 seemed to primarily decrease insulin secretion. In the case of *trans*-nonachlor, decreased insulin synthesis seemed to be more important than decreased insulin secretion. Mechanistically, decreased insulin content in the in vitro experiments may be relevant to the decreased  $\Phi_s$ , whereas the decreased insulin secretion may be relevant to  $\Phi_d$ . Nonetheless, because humans are exposed to all these chemical mixtures, both  $\Phi_d$  and  $\Phi_s$  can be affected by POP mixtures.

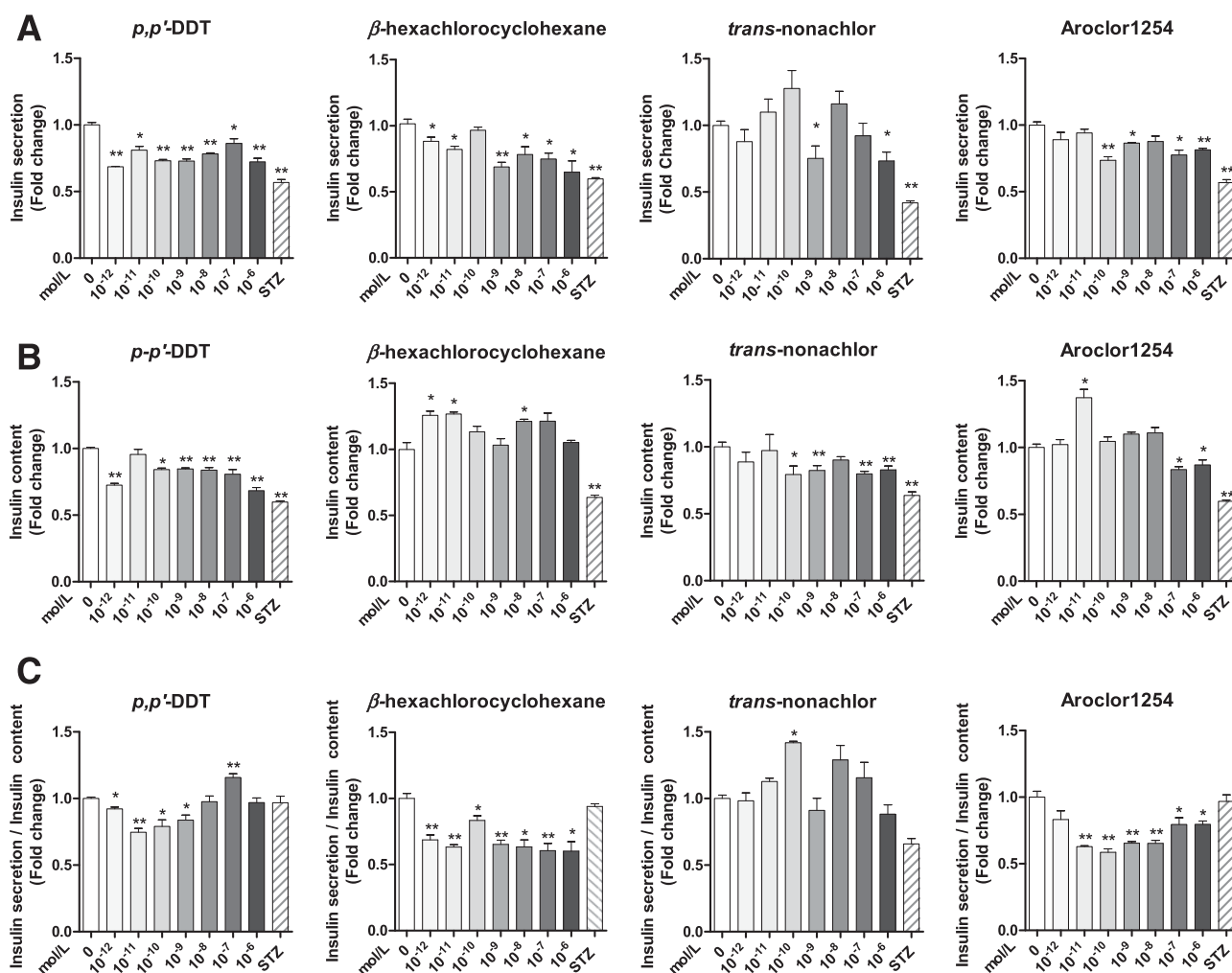
The POP-related decrease in insulin secretion may explain the risk of T2D in Asian and elderly people. Compared with people of European descent, Asians with T2D are characterized by early  $\beta$ -cell dysfunction, which develops at a lower BMI (27). At present, genetic predisposition and a greater tendency for visceral adiposity at any



Table 3—Geometric means of insulin sensitivity and secretion indicators according to categories of obesity-related indices

Category of obesity-related indices							
	Q1 (<25%)	Q2 (25 to <50%)	Q3 (50 to <75%)	Q4-1 (75 to <90%)	Q4-2 (90 to <95%)	Q4-3 (≥95%)	P <sub>trend</sub>
BMI (kg/m <sup>2</sup> )	17.0–22.3	22.4–24.2	24.2–26.4	26.4–28.2	28.2–30.7	30.8–37.4	
n	50	50	50	30	10	10	
S <sub>I</sub> (10 <sup>−4</sup> dL/kg/min per μU/mL)							
Model 1	14.33 ± 1.99	9.33 ± 1.30	6.92 ± 0.96	5.62 ± 1.01	4.61 ± 1.43	3.25 ± 1.01	< 0.01
Model 2	12.14 ± 1.72	9.07 ± 1.24	6.87 ± 0.93	6.33 ± 1.12	5.43 ± 1.64	3.32 ± 1.00	< 0.01
Φ <sub>S</sub> (10 <sup>−9</sup> /min)							
Model 1	45.08 ± 5.09	47.20 ± 5.33	40.81 ± 4.61	47.09 ± 6.87	61.21 ± 15.47	34.83 ± 8.80	0.85
Model 2	42.50 ± 4.97	44.64 ± 5.04	39.90 ± 4.45	51.01 ± 7.43	62.24 ± 15.49	34.37 ± 8.52	0.70
Model 3	42.08 ± 4.85	43.49 ± 4.85	40.89 ± 4.51	51.97 ± 7.46	63.37 ± 15.53	35.62 ± 8.70	0.50
Φ <sub>D</sub> (10 <sup>−9</sup> )							
Model 1	349.06 ± 65.71	438.26 ± 82.50	487.63 ± 91.79	541.09 ± 131.50	650.03 ± 273.62	380.33 ± 160.09	0.20
Model 2	350.56 ± 70.76	409.18 ± 79.77	466.81 ± 89.91	538.08 ± 135.35	588.41 ± 252.79	386.81 ± 165.53	0.24
Model 3	348.01 ± 70.24	401.36 ± 78.50	475.34 ± 91.82	545.51 ± 137.29	596.27 ± 256.10	397.17 ± 170.11	0.20
Waist circumference (cm)	59.5–78.0	79.0–84.0	85.0–91.0	91.5–97.0	97.5–99.5	100.0–119.0	
n	47	54	49	31	9	10	
S <sub>I</sub> (10 <sup>−4</sup> dL/kg/min per μU/mL)							
Model 1	16.33 ± 2.22	9.22 ± 1.17	7.85 ± 1.04	4.25 ± 0.71	4.92 ± 1.53	2.49 ± 0.73	< 0.01
Model 2	13.79 ± 2.14	9.17 ± 1.23	8.19 ± 1.10	5.00 ± 0.87	5.27 ± 1.64	2.75 ± 0.81	< 0.01
Φ <sub>S</sub> (10 <sup>−9</sup> /min)							
Model 1	50.42 ± 5.88	45.83 ± 4.99	44.44 ± 5.08	41.80 ± 6.00	42.93 ± 11.44	31.59 ± 7.99	0.10
Model 2	41.37 ± 5.45	44.44 ± 5.03	44.14 ± 5.02	48.13 ± 7.12	49.41 ± 13.07	33.25 ± 8.31	0.96
Model 3	40.88 ± 5.30	43.69 ± 4.87	44.80 ± 5.02	48.96 ± 7.14	50.70 ± 13.21	34.15 ± 8.40	0.86
Φ <sub>D</sub> (10 <sup>−9</sup> )							
Model 1	479.02 ± 91.30	283.03 ± 50.33	545.94 ± 101.91	603.64 ± 141.66	691.21 ± 301.05	339.48 ± 140.27	0.30
Model 2	420.73 ± 92.49	258.23 ± 48.78	533.42 ± 101.21	596.67 ± 147.42	781.66 ± 345.12	328.09 ± 136.89	0.19
Model 3	416.64 ± 91.49	254.67 ± 48.11	539.85 ± 102.38	605.12 ± 149.40	798.29 ± 352.12	335.30 ± 139.78	0.17
Body fat (%) <sup>*</sup>	14.8–25.5	26.3–32.3	32.4–36.9	37.3–41.7	41.9–43.5	44.1–51.3	
n	49	51	46	32	10	9	
S <sub>I</sub> (10 <sup>−4</sup> dL/kg/min per μU/mL)							
Model 1	12.73 ± 1.87	6.73 ± 0.97	8.35 ± 1.27	6.57 ± 1.20	7.99 ± 2.60	4.87 ± 1.67	0.01
Model 2	15.88 ± 2.28	7.14 ± 0.92	5.84 ± 0.90	4.45 ± 0.81	5.47 ± 1.67	3.59 ± 1.16	< 0.01
Φ <sub>S</sub> (10 <sup>−9</sup> /min)							
Model 1	45.29 ± 5.09	37.82 ± 4.16	48.24 ± 5.59	49.65 ± 6.90	29.72 ± 7.39	79.95 ± 20.95	0.24
Model 2	50.57 ± 6.10	38.88 ± 4.19	41.48 ± 5.35	44.78 ± 6.83	27.66 ± 7.10	74.89 ± 20.33	0.89
Model 3	49.05 ± 5.86	39.55 ± 4.21	41.32 ± 5.25	46.58 ± 7.04	28.51 ± 7.22	77.70 ± 20.82	0.67
Φ <sub>D</sub> (10 <sup>−9</sup> )							
Model 1	339.36 ± 64.57	503.17 ± 93.84	478.05 ± 93.87	435.58 ± 102.55	350.12 ± 147.46	902.97 ± 400.87	0.19
Model 2	343.37 ± 72.64	510.96 ± 96.54	425.44 ± 96.12	421.20 ± 112.67	356.70 ± 160.49	878.76 ± 418.16	0.36
Model 3	334.90 ± 71.13	518.29 ± 98.02	424.03 ± 95.71	435.07 ± 116.87	365.76 ± 164.57	905.87 ± 431.23	0.30

Data are mean ± SD unless otherwise indicated. Model 1: crude. Model 2: adjusted for age, sex, cigarette smoking, alcohol consumption, physical activity, total cholesterol, and triglycerides. Model 3: further adjusted for insulin sensitivity. <sup>\*</sup>Sample size for analyses of percent body fat was 197.



**Figure 3**—Effects of OCPs or Aroclor 1254 (PCB mixture) on glucose-stimulated insulin secretion in INS-1E  $\beta$ -cells. Each chemical was incubated with INS-1E cells at concentrations from  $10^{-12}$  to  $10^{-6}$  mol/L for 48 h. Streptozotocin (STZ) at  $2.5 \times 10^{-4}$  mol/L served as a positive control for  $\beta$ -cell damage. At the end of the incubation period, media were collected to measure secreted insulin (A), and the cells were subjected to extraction to measure cellular insulin content (B). Ratios of insulin secretion to insulin content are also presented (C). Data are mean  $\pm$  SE of six independent samples. \* $P < 0.05$ , \*\* $P < 0.001$  vs. control.

given BMI are suspected to contribute to ethnic disparities in T2D (27). However, the levels of many chemicals belonging to the POP class in both humans and the environment are higher in many Asian countries than in Western Europe or North America because emission sources of many POPs in the past several decades have shifted from industrialized countries of the northern hemisphere to less-developed countries in tropical and subtropical regions (28). Thus, chronic exposure to POPs and other chemicals may be the key reason why  $\beta$ -cell dysfunction–dominant T2D is more common among Asians than among Caucasians. Similarly, the role of POPs in the development of T2D may become more important with aging, given that the body's burden of POPs is higher in elderly people than in younger people because lipophilic chemicals like POPs tend to accumulate with age (29). This observation may explain why insulin secretory defects are more prevalent in T2D for older (30) than for middle-aged adults.

A couple of epidemiological studies suggested that traditional risk factors of T2D, including obesity, have varying relations with incident T2D, depending on preceding degrees of IR or pancreatic  $\beta$ -cell dysfunction before diagnosis (4,31). In particular, among elderly people, greater adiposity and significant weight gain have been linked to even lower rates of incident T2D preceded predominantly by  $\beta$ -cell dysfunction (4,31). These puzzling results also can be explained by POPs because adipose tissue is a major storage site for POPs and can play a primarily protective role against the effects of POPs on critical organs. For example, when two elders are exposed to the same amounts of POPs from the environment, the one with less adiposity has an increased chance of POPs reaching pancreatic  $\beta$ -cells (2).

Although HOMA- $\beta$  has been widely used in epidemiological studies to estimate insulin secretion because of its convenience, it may have a limited value for the evaluation

of the effects of POPs and other chemicals on pancreatic  $\beta$ -cell function. When we compared the results of insulin secretion estimated by 2-h seven-sample OGTT with those of HOMA- $\beta$ , the results differed depending on how insulin secretion was measured. Different associations would be expected because correlation coefficients among these markers were not  $>0.3$ . In addition, the clearly contrasting associations between POPs and  $\Phi_d$  by insulin-resistant levels suggest that the accurate analysis of different phases of insulin secretion is important. Furthermore, the interaction between POPs and insulin-resistant levels on  $\Phi_d$  was observed when HOMA-IR, not the marker of insulin sensitivity estimated by 2-h seven-sample OGTT, was used as a marker of insulin sensitivity. This finding may be related to the fact that OGTT provides useful information about glucose tolerance but not insulin sensitivity/IR per se (32,33). All these findings suggest that how insulin secretion and insulin sensitivity are measured in human studies is important.

The study has several limitations. First, it was cross-sectional, which does not allow for determination of a temporal relation. Nonetheless, that pancreatic  $\beta$ -cell function affects serum concentrations of OCPs or PCBs is unlikely. In addition, the supporting evidence from our in vitro experiments suggests that the associations observed in this study may be causal. In a future study, we can evaluate whether POPs affect the course of  $\beta$ -cell function over time. Second, we assessed insulin secretion and insulin sensitivity by using a reduced version of the oral minimal model rather than gold standard techniques, such as the hyperglycemic-euglycemic clamp, for practical reasons. However, the use of OGTT can be preferable for physiological significance, not for simplicity (14).

In conclusion, this study provides important findings that mechanistically support the recent evidence linking POPs and T2D (1). Chronic exposure to low doses of POPs may increase the risk of T2D by primarily affecting pancreatic  $\beta$ -cell function rather than IR. Because the impairment of insulin secretion (rather than impairment of insulin action) is a critical determinant of T2D (34), the influence of POPs on pancreatic  $\beta$ -cell function should be urgently evaluated in a prospective study.

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**Author Contributions.** Y.-M.L. performed the statistical analyses and drafted the human data parts of the manuscript. C.-M.H. and S.P. performed the in vitro experiments and drafted the in vitro data parts of the manuscript. S.-A.K. conducted the experiments on human subjects. T.T. performed the in vitro experiments. Y.-R.Y., D.-J.K., H.-C.K., and H.-B.M. contributed to the discussion. I.-K.L. conceived the hypothesis and edited the manuscript. D.-H.L. conceived the

hypothesis, led the project, and edited the manuscript. D.-H.L. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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